

# Restricting Excessive Cardiac Action Potential and QT Prolongation

## A Vital Role for $I_{Ks}$ in Human Ventricular Muscle

Norbert Jost, PhD; László Virág, PhD; Miklós Bitay, MD, PhD; János Takács, MD, PhD; Csaba Lengyel, MD, PhD; Péter Biliczki, MD; Zsolt Nagy, MSc; Gábor Bogáts, MD, PhD; David A. Lathrop, PhD; Julius G. Papp, MD, DSc; András Varró, MD, DSc

**Background**—Although pharmacological block of the slow, delayed rectifier potassium current ( $I_{Ks}$ ) by chromanol 293B, L-735,821, or HMR-1556 produces little effect on action potential duration (APD) in isolated rabbit and dog ventricular myocytes, the effect of  $I_{Ks}$  block on normal human ventricular muscle APD is not known. Therefore, studies were conducted to elucidate the role of  $I_{Ks}$  in normal human ventricular muscle and in preparations in which both repolarization reserve was attenuated and sympathetic activation was increased by exogenous dofetilide and adrenaline.

**Methods and Results**—Preparations were obtained from undiseased organ donors. Action potentials were measured in ventricular trabeculae and papillary muscles using conventional microelectrode techniques; membrane currents were measured in ventricular myocytes using voltage-clamp techniques. Chromanol 293B (10  $\mu$ mol/L), L-735,821 (100 nmol/L), and HMR-1556 (100 nmol/L and 1  $\mu$ mol/L) produced a <12-ms change in APD while pacing at cycle lengths ranging from 300 to 5000 ms, whereas the  $I_{Kr}$  blockers sotalol and E-4031 markedly lengthened APD. In voltage-clamp experiments, L-735,821 and chromanol 293B each blocked  $I_{Ks}$  in the presence of E-4031 to block  $I_{Kr}$ . The E-4031-sensitive current ( $I_{Kr}$ ) at the end of a 150-ms-long test pulse to 30 mV was  $32.9 \pm 6.7$  pA ( $n=8$ ); the L-735,821-sensitive current ( $I_{Ks}$ ) magnitude was  $17.8 \pm 2.94$  pA ( $n=10$ ). During a longer 500-ms test pulse,  $I_{Kr}$  was not substantially changed ( $33.6 \pm 6.1$  pA;  $n=8$ ), and  $I_{Ks}$  was significantly increased ( $49.6 \pm 7.24$  pA;  $n=10$ ). On application of an “action potential-like” test pulse,  $I_{Kr}$  increased as voltage became more negative, whereas  $I_{Ks}$  remained small throughout all phases of the action potential-like test pulse. In experiments in which APD was first lengthened by 50 nmol/L dofetilide and sympathetic activation was increased by 1  $\mu$ mol/L adrenaline, 1  $\mu$ mol/L HMR-1556 significantly increased APD by  $14.7 \pm 3.2\%$  ( $P<0.05$ ;  $n=3$ ).

**Conclusions**—Pharmacological  $I_{Ks}$  block in the absence of sympathetic stimulation plays little role in increasing normal human ventricular muscle APD. However, when human ventricular muscle repolarization reserve is attenuated,  $I_{Ks}$  plays an increasingly important role in limiting action potential prolongation. (*Circulation*. 2005;112:1392-1399.)

**Key Words:** arrhythmia ■ electrophysiology ■ ion channels ■ long-QT syndrome ■ potassium channels

Action potential repolarization is an important phenomenon in the heart where it controls action potential duration (APD) and thus affects refractoriness and conduction of electrical impulses throughout the heart. Several pharmacological agents intended to abate cardiac arrhythmias specifically target mechanisms that regulate APD. In the ventricles, APD, in large part, determines dynamic changes in QT duration on a beat-by-beat basis.

### See p 1376

The rapid component of the delayed rectifier potassium current ( $I_{Kr}$ ) has been identified in several mammalian spe-

cies,<sup>1-3</sup> including humans,<sup>4-7</sup> and pharmacological agents that selectively block  $I_{Kr}$  (eg, E-4031, sotalol, and dofetilide) markedly increase APD, QT duration, and ventricular refractoriness. As such, high doses of these  $I_{Kr}$  blockers are associated with induction of torsade de pointes.<sup>8,9</sup> In addition, mutations in ion channel genes (eg, HERG and KCNE2) that suppress  $I_{Kr}$  are associated with the inherited long-QT syndrome LQT2, which is linked to an increased incidence of sudden cardiac death.<sup>10</sup> As such,  $I_{Kr}$  plays a major role in regulation of action potential repolarization and is important in the maintenance of normal heart rhythms; loss of this current is highly arrhythmogenic.<sup>11</sup>

Received March 17, 2005; revision received July 7, 2005; accepted July 11, 2005.

From the Department of Pharmacology and Pharmacotherapy (N.J., L.V., J.T., P.B., Z.N., J.G.P., A.V.), Department of Cardiac Surgery (M.B., G.B.), and First Department of Internal Medicine (C.L.), University of Szeged, and Division for Cardiovascular Pharmacology, Hungarian Academy of Sciences (N.J., J.G.P.), Szeged, Hungary, and Heart Research Program (D.A.L.), National Heart, Lung and Blood Institute, Bethesda, Md.

Correspondence to Professor András Varró, Department of Pharmacology and Pharmacotherapy, University of Szeged, H-6720 Szeged, Dóm tér 12, PO Box 427, Hungary. E-mail a.varro@phcol.szote.u-szeged.hu

© 2005 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/CIRCULATIONAHA.105.550111

The role of the slow delayed rectifier potassium current ( $I_{Ks}$ ) in human ventricular muscle action potential repolarization, on the other hand, has been debated. As with  $I_{Kr}$ ,  $I_{Ks}$  has been identified in several mammalian species,<sup>1-3</sup> including humans,<sup>6,12</sup> and mutations in *KCNQ1* and *KCNE1*, the  $\alpha$ - and  $\beta$ -subunits of the  $I_{Ks}$  potassium channel, are associated with another specific form of the inherited long-QT syndrome, LQT1.<sup>10</sup> However, we have previously demonstrated that complete pharmacological block of  $I_{Ks}$  by either chromanol 293B or L-735,821 has little effect on APD in isolated dog and rabbit ventricular muscle<sup>11,13</sup> over a wide range of physiological pacing frequencies. These findings led us to speculate that  $I_{Ks}$  normally plays little role in ventricular muscle action potential repolarization, but when APD is abnormally long,  $I_{Ks}$  likely provides an important safety mechanism that, when removed, increases arrhythmic risk.<sup>11,13</sup> Other investigators have confirmed these previous findings,<sup>14</sup> and computer simulations supported speculation that  $I_{Ks}$  plays little role in adaptations of APD to changes in heart rate.<sup>15</sup> The role of  $I_{Ks}$  in human ventricular muscle, however, remains controversial despite our preliminary characterizations of  $I_{Kr}$ <sup>7</sup> and  $I_{Ks}$ <sup>12</sup> in isolated human ventricular myocytes that suggest that both currents behave similarly as in isolated dog<sup>2,11</sup> and rabbit<sup>3,13</sup> ventricular myocytes. Thus, the purpose of the present study was to confirm our initial findings while elucidating the role of  $I_{Ks}$  in normal human ventricular muscle action potential repolarization in the absence of tonic sympathetic stimulation.

Preliminary results of this work were presented at the 1999 Annual Scientific Session of the American Heart Association.<sup>16</sup>

## Methods

### Patients

Hearts obtained from organ donors were explanted to obtain pulmonary and aortic valves for transplant surgery. Before cardiac explantation, organ donor patients did not receive medication except dobutamine, furosemide, and plasma expanders. The investigations conform to the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997, 35:2 to 35:4), and all experimental protocols were approved by the Albert Szent-Györgyi Medical University Ethical Review Board (No. 51-57/1997 OEj). Proper consent was obtained for use of each individual's tissue for experimentation.

### Action Potential Measurements in Multicellular Preparations

#### Tissue Preparation

Action potentials were recorded in ventricular trabeculae and papillary muscle preparations (<2 mm in diameter; n=45) obtained from the right ventricles of 26 undiseased human donor hearts (from 17 men, 9 women; age,  $41.7 \pm 4.1$  years) using conventional microelectrode techniques. After explantation, each heart was perfused with cardioplegic solution and kept cold (4°C to 6°C) for 2 to 4 hours before dissection. Trabeculae and papillary muscles were then excised and mounted in a tissue chamber (volume,  $\approx 50$  mL) perfused with oxygenated (95%  $O_2$ +5%  $CO_2$ ) modified Tyrode's solution containing (in mmol/L) NaCl 115, KCl 4,  $CaCl_2$  1.8, MgCl 1,  $NaHCO_3$  20, and glucose 11. The pH of this solution was 7.35 to 7.45 at 37°C.

#### Protocol

Initially, each preparation was stimulated at a basic cycle length of 1000 ms (frequency, 1 Hz) using 2-ms-long rectangular constant-voltage pulses isolated from ground and delivered via bipolar platinum electrodes in contact with the preparation using an EMG

4767 type of stimulator (Medicor Ltd). One hour or more was allowed for each preparation to equilibrate while continuously superfused with Tyrode's solution warmed to 37°C. Transmembrane potentials were recorded using a conventional glass microelectrode filled with 3 mol/L KCl with a tip resistance of 5 to 20 mol/L $\Omega$  connected to an high-impedance electrometer (Bio-Logic VF102, CLAIX, F-38640) referenced to ground. The first derivative of transmembrane potential ( $V_{max}$ ) was electronically obtained using a Bio-Logic DV-140 (Claix, F-38640) differentiator designed and calibrated to have a linear response over the range of 10 to 1000 V/s. Amplifier outputs were digitized with an ADA 3300 analog-to-digital converter (Real Time Devices Inc) with a maximum sampling frequency of 50 kHz connected to an IBM-compatible personal computer. Data were stored and analyzed on a personal computer while being monitored on a dual-beam memory oscilloscope (Tektronix 2230). Resting membrane potential, action potential amplitude, and APD, measured at 50% and 90% repolarization (APD<sub>50</sub> and APD<sub>90</sub>), were automatically measured with software developed in our laboratory (APES, Hugo Sachs Elektronik, March-Hugstetten). Stimulation pulses were also controlled by PC software providing constant-current pulses with programmed timing and amplitudes to the preparation via a EMG 47671 type of signal isolator (Medicor Ltd, Budapest).

In each experiment, baseline action potential characteristics were first obtained during superfusion with normal 37°C Tyrode's solution during continuous pacing at a basic cycle length of 1000 ms, followed by a run of changing pacing cycle lengths (300, 400, 500, 700, 1000, 1500, 2000, 3000, and 5000 ms) sequentially applied for 25 beats each. This procedure allowed action potential parameters to be quickly obtained at each pacing cycle length after a "quasi-steady state" was established. Recordings were continuously monitored to confirm 1-to-1 activation throughout the procedure.

After baseline measurements were obtained, each preparation was superfused with Tyrode's solution containing a single test drug diluted to the proper concentration for 40 to 60 minutes before measurements were repeated at 3-minute intervals in the continued presence of the test drug until a <5% change occurred in action potential characteristics between subsequent samples. When microelectrode impalement was lost, reimpalement was attempted. If action potential characteristics recorded with the new impalement deviated by >5% from the preceding ones, the experiment was terminated, and results were excluded from evaluation.

### Drugs Used

The  $I_{Kr}$  blockers d-sotalol (Bristol-Myers Squibb Co) and E-4031 (Institute for Drug Research Ltd) were prepared daily from aqueous stock solutions (30 and 10 mmol/L, respectively). Similarly, the  $I_{Ks}$  blockers chromanol 293B (Aventis Pharma) and HMR-1556 (Aventis Pharma) plus L-735,821 (Merck-Schaepe & Dohme Co) were diluted from stock solutions (10 and 1 mmol/L, respectively) containing 100% dimethyl sulfoxide. This procedure resulted in a 0.1% dimethyl sulfoxide concentration when the effects of the drugs were examined. This and the lower dimethyl sulfoxide concentrations alone did not affect action potential characteristics in separate studies.

### Transmembrane Current Measurements

#### Cell Isolation

Ventricular myocytes were enzymatically dissociated from 7 human hearts (3 male donors, 4 female donors; age,  $41.55 \pm 5.7$  years). After explantation, hearts were transported to the laboratory in cold (4°C) cardioplegic solution. A portion of the left ventricular wall was excised with an attached branch of the left descending coronary artery, which was cannulated and mounted on a modified 60-cm-high Langendorff perfusion apparatus. Each preparation was perfused with each of the following perfusates for the times indicated: (1) normal Tyrode's solutions, 10 minutes; (2)  $Ca^{2+}$ -free Tyrode's solution, 10 minutes, and (3)  $Ca^{2+}$ -free Tyrode's solution containing collagenase (type I, 0.66 mg/mL, Sigma-Aldrich Fine Chemicals), elastase (type III, 0.045 mg/mL, Sigma-Aldrich Fine Chemicals), taurine (50 mmol/L, Sigma-Aldrich Fine Chemicals), and BSA (fraction V, fatty acid free, 2 mg/mL, Sigma-Aldrich Fine Chemi-

cals), 15 minutes. After this series of perfusions, the dissociation solution was supplemented with protease (type XIV, 0.12 mg/mL, Sigma-Aldrich Fine Chemicals), and the tissue was perfused for an additional 45 to 60 minutes. Portions of clearly digested left ventricular wall were removed, minced into small pieces, and placed into either Kraft-Brühe (KB) solution<sup>17</sup> or  $\text{Ca}^{2+}$ -free Tyrode's solution supplemented  $\text{CaCl}_2$  (1.25 mmol/L) for 15 minutes. After this period, the suspension of minced tissue was gently agitated in a small beaker to obtain single cells. Although myocytes thus obtained were of epicardial, midmyocardial, and endocardial origin, there was no way to determine from which an individual cell was dissociated.

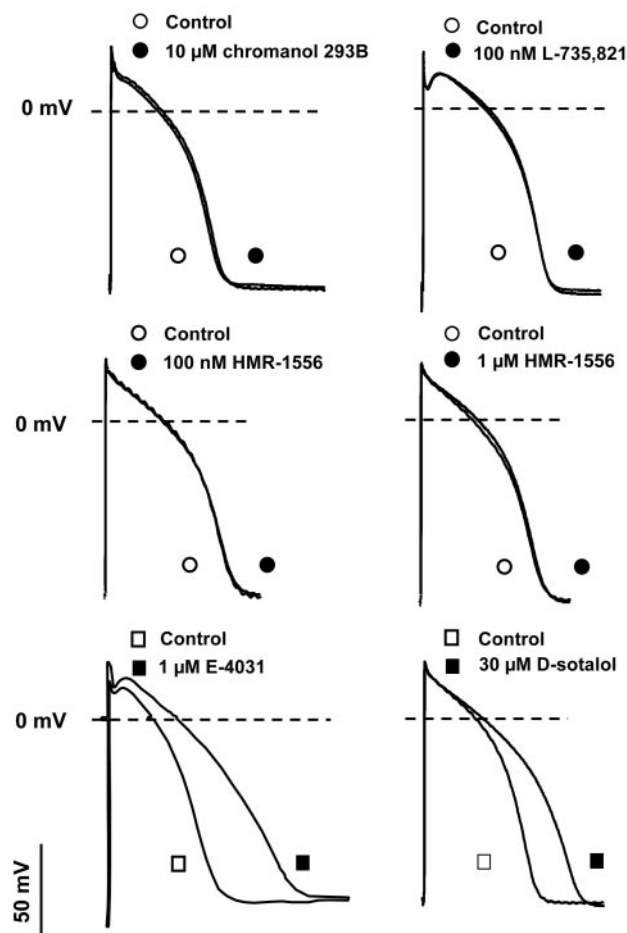
Throughout the isolation procedure, all solutions were oxygenated with 100%  $\text{O}_2$  while their temperatures were maintained at 37°C. After a suspension of isolated myocytes was obtained, individual heart cells were allowed to settle to the bottom of the beaker for 10 minutes. Half of the supernatant was then replaced by fresh solution, and the cells resuspended in the solution were allowed to settle. This procedure was repeated 3 times to retard further enzymatic digestion. Myocytes in KB solution were stored at 4°C, and those stored in Tyrode's solution were maintained at 12°C to 14°C.

### Cell Isolation Solutions

The composition of each solution used for myocyte isolation was (in mmol/L) as follows: (1) cardioplegic solution: NaCl 110, KCl 16,  $\text{MgCl}_2$  16,  $\text{CaCl}_2$  1.2, and  $\text{NaHCO}_3$  10; (2) normal Tyrode's solution: NaCl 135, KCl 4.7,  $\text{NaHCO}_3$  4.4,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{MgSO}_4$  1.2, HEPES 10, glucose 10, and  $\text{CaCl}_2$  1.0 (pH 7.2 adjusted with NaOH); (3)  $\text{Ca}^{2+}$ -free solution: NaCl 135, KCl 4.7,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{MgSO}_4$  1.2, HEPES 10,  $\text{NaHCO}_3$  4.4, and glucose 10 (pH 7.2 adjusted with NaOH); and (4) KB solution: KOH 90, L-glutamic acid 70, taurine 15, KCl 30,  $\text{KH}_2\text{PO}_4$  10,  $\text{MgCl}_2$  0.5, HEPES 10, glucose 11, and EGTA 0.5 (pH 7.3 adjusted with KOH).

### Experimental Protocol

One drop of the myocyte suspension was placed in a 0.4-mL transparent recording chamber mounted on the stage of an inverted microscope (TMS, Nikon UK Ltd), and at least 5 minutes was allowed for individual myocytes to settle and adhere to its bottom before superfusion was initiated. Only rod-shaped, clearly striated myocytes (average length, 100  $\mu\text{m}$ ; diameter, 20  $\mu\text{m}$ ;  $C_m$ , 210  $\mu\text{F}$ ) were used for recording. Although the yield varied greatly (from 5% to 70%) between each dissociation procedure, the ease of electrode-seal formation and the quality of the measurements obtained did not correlate with yield. HEPES-buffered Tyrode's solution was used as the normal superfusate. This solution contained (in mmol/L) NaCl 144,  $\text{NaH}_2\text{PO}_4$  0.33, KCl 4.0,  $\text{CaCl}_2$  1.8,  $\text{MgCl}_2$  0.53, glucose 5.5, and HEPES 5.0 at pH 7.4. Superfusion was maintained by gravity flow at  $\approx 2$  to 2.5 mL/min. Micropipettes were fabricated from borosilicate glass capillaries (Clark Electromedical Instruments) using a microprocessor-controlled horizontal puller (Sutter Instruments). These electrodes had resistances between 1.5 and 2.5 M $\Omega$  when filled with a pipette solution containing (in mmol/L) K-aspartate 100, KCl 20, MgATP 5, K<sub>2</sub>BAPTA 5, HEPES 10, and glucose 5. The pH of the filling solution was adjusted to 7.2 by KOH. BAPTA rather than EGTA was used in the pipette solution to minimize the possible influence of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger current. The external solution contained 1  $\mu\text{mol/L}$  nisoldipine to completely block the inward  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ). The inward sodium current ( $I_{\text{Na}}$ ) was inactivated using a  $-40$ -mV holding potential, which also largely inactivated the transient outward current ( $I_{\text{to}}$ ). Membrane currents were recorded with either an Axopatch-1D or an Axopatch 200B patch-clamp amplifier (Axon Instruments) in the whole-cell patch-clamp configuration. After a high- ( $1$  to  $10$  G $\Omega$ ) resistance seal was established by gentle suction, the cell membrane beneath the electrode tip was disrupted by additional suction or application of 1.5-V electrical pulses for 1 to 5 ms. Series resistance was typically 4 to 8 M $\Omega$  before compensation (usually 50% to 80%). When the initial series resistance was  $>10$  M $\Omega$  or when series resistance increased substantially during measurement, the experiment was terminated, and the results were excluded from analyses. Membrane currents were digitized with a 333-kHz analog-to-digital converter



**Figure 1.** Human ventricular papillary muscle action potential recordings in the absence of any sympathetic agonist before and after 40 minutes of superfusion with the  $I_{\text{Ks}}$  blockers 10  $\mu\text{mol/L}$  chromanol 293B (top left), 100 nmol/L L-735,821 (top right), and 100 nmol/L and 1  $\mu\text{mol/L}$  HMR-1556 (middle) and the  $I_{\text{Kr}}$  blockers 1  $\mu\text{mol/L}$  E-4031 (bottom left) and 30  $\mu\text{mol/L}$  d-sotalol (bottom right). Stimulation frequency was 1 Hz.

(Digidata 1200, Axon Instruments) under software control (pClamp 6.0 and pClamp7, Axon Instruments), and results were analyzed with Axon software programs (pClamp 6.0 and pClamp7.0, Axon Instruments) after low-pass (1-kHz) filtration.

### Statistical Analysis

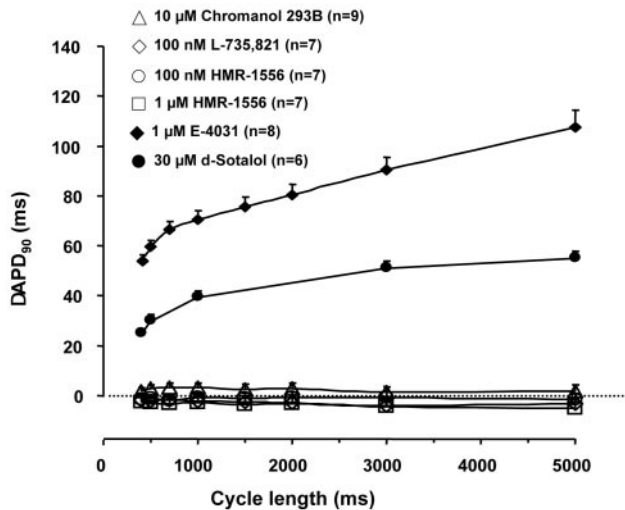
Student *t* tests for paired data were used to compare results. Results were considered significant at  $P < 0.05$ .

## Results

### Effects of $I_{\text{Ks}}$ and $I_{\text{Kr}}$ Blockade on Human Ventricular Muscle APD

Concentrations of chromanol 293B (10  $\mu\text{mol/L}$ ), L-735,821 (100 nmol/L), and HMR-1556 (100 nmol/L and 1  $\mu\text{mol/L}$ ) reported to selectively block  $I_{\text{Ks}}$  in cardiac ventricular muscle preparations<sup>11–14,18,19</sup> in other species produced a  $<9$ -ms (2.8%) change in human ventricular papillary muscle APD after 40 minutes of exposure during continuous pacing at a cycle length of 1000 ms (Figure 1, top and middle). In contrast, concentrations of d-sotalol (30  $\mu\text{mol/L}$ ) and E-4031 (1  $\mu\text{mol/L}$ ), expected to selectively block  $I_{\text{Kr}}$ <sup>11</sup>, markedly and





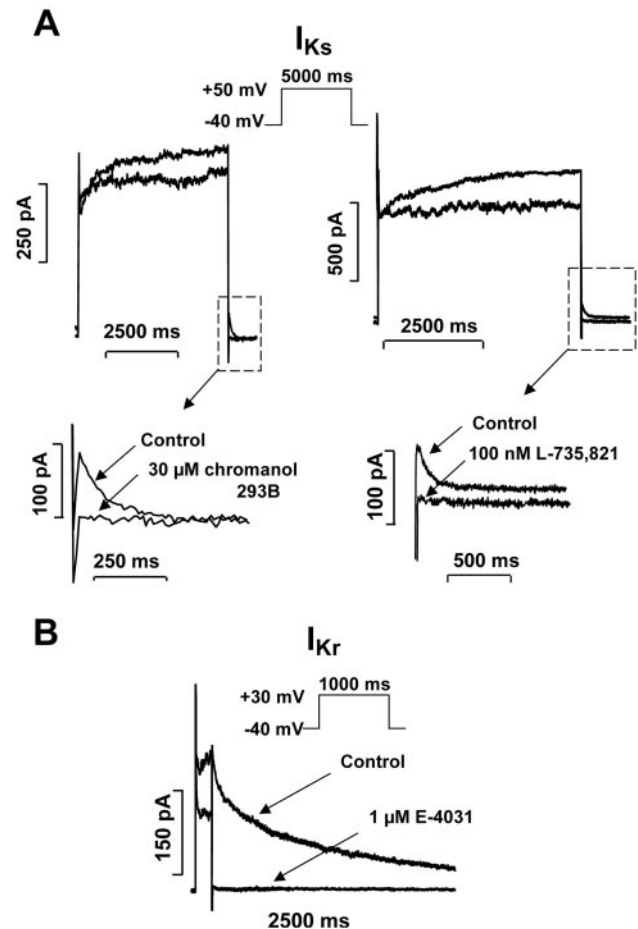
**Figure 2.** Frequency-dependent effect of  $I_{Kr}$  (by 1  $\mu\text{mol/L}$  E-4031 or 30  $\mu\text{mol/L}$  sotalol) and  $I_{Ks}$  block (by 10  $\mu\text{mol/L}$  chromanol 293B, 100 nmol/L and 1  $\mu\text{mol/L}$  HMR-1556, or 100 nmol/L L-735,821) on APD in canine ventricular papillary muscles in the absence of any sympathetic agonist. Bars represent mean  $\pm$  SEM.

significantly increased human ventricular muscle APD under identical conditions (Figure 1, bottom). This difference in the effects of chromanol 293 B (10  $\mu\text{mol/L}$ ), L-735,821 (100 nmol/L), and HMR 1556 (100 nmol/L and 1  $\mu\text{mol/L}$ ) compared with d-sotalol (30  $\mu\text{mol/L}$ ) and E-4031 (1  $\mu\text{mol/L}$ ) on APD was observed in human ventricular muscle over a wide range of pacing cycle lengths (300 to 5000 ms) (Figure 2). Over this range of pacing cycle lengths, chromanol 293B (Figure 2), L-735,821, or HMR (100 nmol/L and 1  $\mu\text{mol/L}$ ) produced a change of  $\leq 12$  ms (3.2%) in APD, whereas d-sotalol and E-4031 each markedly lengthened human ventricular APD in a reverse frequency-dependent manner.

### $I_{Kr}$ and $I_{Ks}$ Characterization in Undiseased Human Ventricular Myocytes

Figure 3A illustrates the effects of 100 nmol/L L-735,821 and 30  $\mu\text{mol/L}$  chromanol 293B on  $I_{Ks}$  tail currents in isolated human ventricular myocytes after 5000-ms-long test pulses to between 0 and 50 mV from and return to a holding potential of  $-40$  mV in the presence of 1  $\mu\text{mol/L}$  E-4031. E-4031 was added to completely inhibit  $I_{Kr}$ . L-735,821 (100 nmol/L) completely abolished  $I_{Ks}$ , whereas chromanol 293B (30  $\mu\text{mol/L}$ ) nearly did so. In other experiments, the selective  $I_{Kr}$  E-4031 (1  $\mu\text{mol/L}$ ) alone completely blocked  $I_{Kr}$  tail currents (Figure 3B) elicited by 1000-ms-long test pulses to between  $-20$  and 50 mV from and to return to a holding potential of  $-40$  mV.

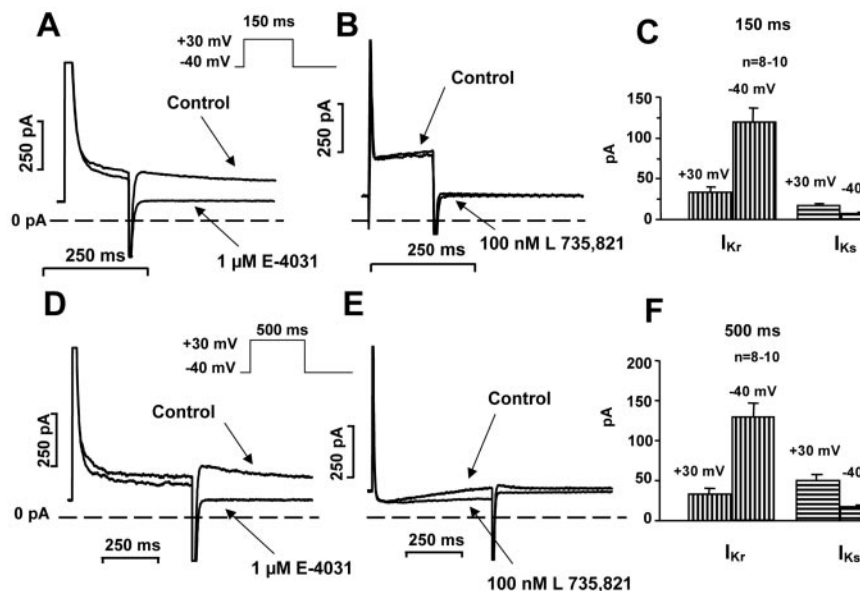
The relative magnitude of  $I_{Ks}$  and  $I_{Kr}$  activated during a standardized human ventricular action potential depolarization was estimated by comparing the amplitudes of the L-735,821-sensitive ( $I_{Ks}$ ) and E-4031-sensitive ( $I_{Kr}$ ) currents at the end of a 150-ms-long test pulse to 30 mV and their tail currents on return to a  $-40$  mV holding potential. This test potential amplitude is slightly positive to the normal human ventricular muscle action potential plateau, whereas the holding potential used is representative of a voltage encoun-



**Figure 3.**  $I_{Kr}$  and  $I_{Ks}$  in human ventricular myocytes in the absence of any sympathetic agonist. A, Recordings in the absence and presence of 10  $\mu\text{mol/L}$  chromanol 293B (left) or 100 nmol/L L-735,821 (right). Nisoldipine (1  $\mu\text{mol/L}$ ) was used to block inward calcium current ( $I_{Ca}$ ) and E-4031 (5  $\mu\text{mol/L}$ ) to block  $I_{Kr}$ . Holding potential is  $-40$  mV; pulse duration, 5000 ms; and pulse frequency, 0.1 Hz. B, Recordings in the absence and presence of 1  $\mu\text{mol/L}$  E-4031. Nisoldipine (1  $\mu\text{mol/L}$ ) was used to block  $I_{Ca}$  and L-735,821 (100 nmol/L) to block  $I_{Ks}$ . Holding potential is  $-40$  mV; pulse duration, 1000 ms; and pulse frequency, 0.05 Hz.

tered during final action potential repolarization. Under these conditions,  $I_{Ks}$  and  $I_{Kr}$  were measured by subtracting membrane currents before and after 4 to 5 minutes of exposure to L-735,821 (100 nmol/L) or E-4031 (1  $\mu\text{mol/L}$ ), respectively. The E-4031-sensitive current ( $I_{Kr}$ ) amplitude at the end of the 150-ms-long test pulse was  $32.9 \pm 6.7$  pA ( $n=8$ ) or  $\approx 27\%$  of the tail current amplitude measured after the voltage test pulse returned to  $-40$  mV ( $119.9 \pm 16.6$  pA;  $n=8$ ) (Figure 4A and 4C). Under identical voltage-clamp conditions, the L-735,821-sensitive current ( $I_{Ks}$ ) during the test pulse to 30 mV was larger than its tail current on return to  $-40$  mV (Figure 4B and 4C). The magnitude of  $I_{Ks}$  during the test pulse was  $17.8 \pm 2.94$  pA at 30 mV compared with  $6.7 \pm 1.93$  pA at  $-40$  mV ( $n=10$ ) and approximately an order of magnitude less than the  $I_{Kr}$  tail current.

When  $I_{Ks}$  and  $I_{Kr}$  were individually measured after 500-ms-long test pulses to 30 mV and their values were compared with those obtained above after a 150-ms test pulse to the



**Figure 4.** E-4031- ( $I_{Kr}$ ; A) and L-735,821- ( $I_{Ks}$ ; B) sensitive currents in human ventricular myocytes after application of a short (150 ms) depolarizing test pulse to 30 mV from a holding potential of -40 mV in the absence of any sympathetic agonist. Recordings of E-4031- ( $I_{Kr}$ ; D) and L-735,821- ( $I_{Ks}$ ; E) sensitive currents after a long (500 ms) depolarizing test pulse to 30 mV from a holding potential of -40 mV. Average  $I_{Kr}$  and  $I_{Ks}$  currents at the end of short (150 ms; C) and long (500 ms; F) depolarizing test pulses to 30 mV, and peak tail current at -40 mV. Bars represent mean  $\pm$  SEM.

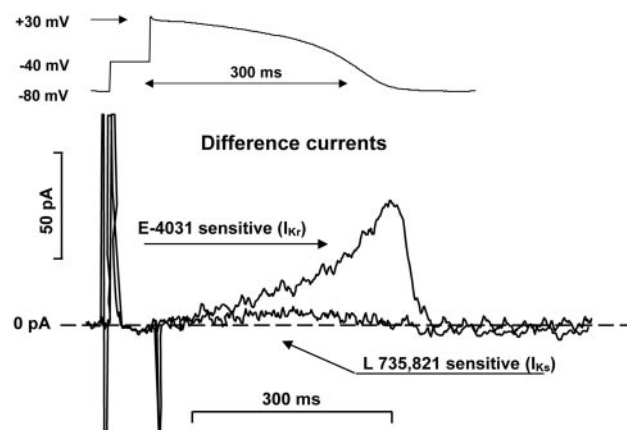
same potential at the same frequency,  $I_{Kr}$  was not substantially changed ( $33.6 \pm 6.1$  pA at 30 mV and  $128.1 \pm 17.4$  pA at -40 mV;  $n=8$ ) (Figure 4D and 4F). This lack of effect of increasing test pulse duration on  $I_{Kr}$  in human ventricular myocytes was similar to that which we previously reported in rabbit and dog. This failure to influence  $I_{Kr}$  occurred because  $I_{Kr}$  activated completely and quickly before the end of either the 150- or 500-ms test pulse. The magnitude of  $I_{Ks}$ , however, was significantly increased when the test pulse duration was increased from 150 to 500 ms ( $49.6 \pm 7.24$  pA at 30 mV and  $16.4 \pm 3.0$  pA at -40 mV;  $n=10$ ). This increase in  $I_{Ks}$  occurred because  $I_{Ks}$  activated slowly so that it continued to activate beyond the shorter 150-ms test pulse duration (Figure 4E and 4F).

To determine the relative roles of  $I_{Ks}$  and of  $I_{Kr}$  under more physiological conditions, we also compared their magnitudes while applying an idealized ventricular muscle "action potential-like" test pulse. The test pulse used for these experiments was obtained by digitizing a representative human right ventricular action potential recorded using the conventional microelectrode technique during continuous pacing at a cycle length of 1000 ms. The waveform thus obtained arose from a diastolic potential of -85 mV and was 115 mV in amplitude with an APD<sub>90</sub> of 300 ms, which roughly corresponds to a normal human QT duration of 0.40 seconds at a heart rate of 70 bpm. A 40-ms-long prepulse to -40 mV was added (Figure 5, inset) to the beginning of this idealized action potential. When the  $I_{Kr}$  difference current (ie, the E-4031-sensitive current) was measured during the idealized action potential plateau, applied as a voltage-clamp test pulse, it was small and increased in magnitude as the test voltage became more negative (Figure 5). In contrast,  $I_{Ks}$ , measured as the L-735,821-sensitive current, remained small throughout all phases of the action potential-like test pulse (Figure 5). Similar results were obtained in 4 additional human right ventricular myocytes prepared from 3 additional normal donor hearts. These results again indicate that the outward current normally carried by  $I_{Kr}$  throughout the ventricular

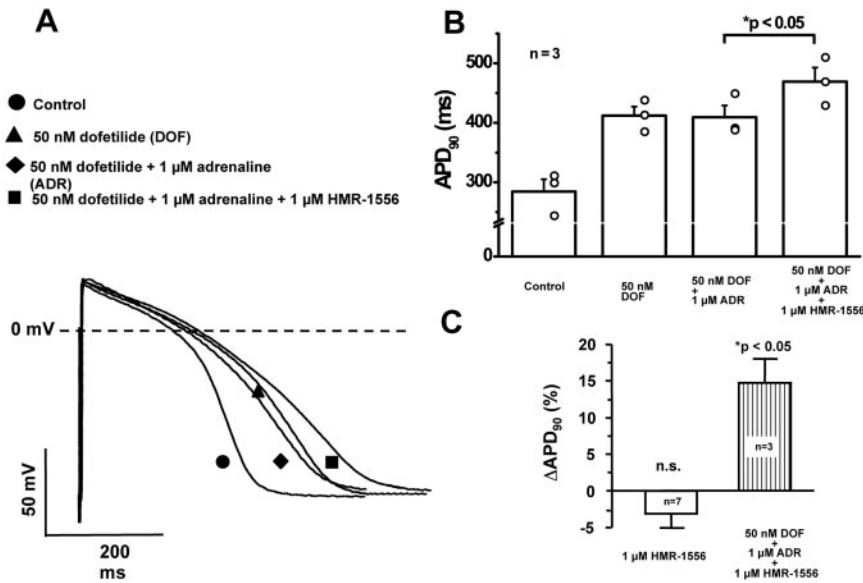
action potential is considerably larger than that carried by  $I_{Ks}$  in the absence of sympathetic agonists. These results are consistent our finding that  $I_{Ks}$  block by chromanol 293B (10  $\mu$ mol/L), L-735,821 (100 nmol/L), or HMR-1556 (100 nmol/L and 1  $\mu$ mol/L) produces little effect on APD, whereas  $I_{Kr}$  block by d-sotalol (30  $\mu$ mol/L) or E-4031 (1  $\mu$ mol/L) greatly increased APD (Figure 1).

#### Effect of $I_{Ks}$ Block During Increased Sympathetic Activation After Attenuation of Repolarization Reserve

The effect of 1  $\mu$ mol/L HMR-1556 was also tested in preparations in the presence of 1  $\mu$ mol/L adrenaline and 50 nmol/L dofetilide. In these experiments, HMR-1556-induced  $I_{Ks}$  block significantly lengthened APD ( $14.7 \pm 3.2\%$ ;



**Figure 5.** E-4031- (1  $\mu$ mol/L) sensitive ( $I_{Kr}$ ) and L-735,821- (100 nmol/L) sensitive ( $I_{Ks}$ ) difference currents recorded during an action potential-like test pulse in human ventricular myocytes in the absence of any sympathetic agonist. The action potential-like test pulse was obtained by recording a normal human ventricular action potential with a conventional microelectrode in a multicellular human papillary muscle preparation and adding a 50-ms prepulse from -80 to -40 mV. Similar results were obtained in 4 additional myocytes.



**Figure 6.** Effect of 1  $\mu\text{mol/L}$  HMR-1556 on human ventricular action potentials recorded in the presence of 50 nmol/L dofetilide (DOF) and 1  $\mu\text{mol/L}$  adrenaline (ADR). A, Representative action potentials recorded at baseline (●), after exposure to 50 nmol/L dofetilide (▲) and 50 nmol/L dofetilide plus 1  $\mu\text{mol/L}$  adrenaline (◆), and after addition of 1  $\mu\text{mol/L}$  HMR-1556 in the continued presence of dofetilide and adrenaline (■). B, Similar effects on APD averaged in 3 experiments. Bars show mean  $\pm$  SEM; circles represent each individual measurement. C, Comparison of the effect of 1  $\mu\text{mol/L}$  HMR-1556 on normal and on action potentials recorded in the presence of 50 nmol/L dofetilide and 1  $\mu\text{mol/L}$  adrenaline. The open bar indicates the APD change observed after 1  $\mu\text{mol/L}$  HMR-1556 in normal preparations; the striated bar shows changes in APD evoked by HMR-1556 in preparations in which sympathetic activation was first increased by 1  $\mu\text{mol/L}$  adrenaline and

repolarization reserve was reduced by 50 nmol/L dofetilide. Columns and error bars indicate mean  $\pm$  SEM,  $n=3$  between the conditions represented by the bars.

$P<0.05$ ;  $n=3$ ; Figure 6). This effect is in sharp contrast to the negligible effect of HMR-1556 on normal APD (Figures 1 and 2) and indicates that the effect of  $I_{Ks}$  on repolarization is substantially increased when sympathetic activation is increased and when a reduction in repolarization reserve results in an abnormally long APD.

## Discussion

Our results indicate that in isolated, multicellular ventricular muscle obtained from normal, undiseased human hearts, chromanol 293B, L-735,821, and HMR-1556 did not markedly increase action potential over a range of pacing cycle lengths corresponding to heart rates of 12 to 200 bpm in the absence of a sympathetic agonist. In addition, our studies indicate that the concentrations of chromanol 293B and L-735,821 (10  $\mu\text{mol/L}$  and 100 nmol/L, respectively) that failed to increase APD significantly blocked  $I_{Ks}$  in ventricular myocytes isolated from the same normal, undiseased human hearts. In contrast to these findings, we demonstrated that E-4031 (1  $\mu\text{mol/L}$ ) blocked  $I_{Kr}$  and dramatically increased normal human ventricular muscle APD, as did sotalol (30  $\mu\text{mol/L}$ ), another recognized  $I_{Kr}$  blocker that also dramatically increased human ventricular muscle APD under the same conditions in which chromanol 293B and L-735,821 failed.

The only study<sup>18</sup> before this one to describe the effect of chromanol on human ventricular action potential characteristics was performed in right ventricular myocytes isolated from hearts explanted from patients with end-stage heart failure. In that study,<sup>18</sup> 1 to 10  $\mu\text{mol/L}$  chromanol 293B was reported to significantly increase APD. However, when Schrieck et al<sup>19</sup> examined the effects of 10  $\mu\text{mol/L}$  chromanol in guinea pig ventricular muscle preparations, they found no effect on APD in the absence of  $\beta$ -adrenoceptor stimulation.<sup>19</sup> When we previously examined the effects of chromanol 293B in rabbit<sup>13</sup> and dog<sup>11</sup> ventricular papillary muscle preparations, 10  $\mu\text{mol/L}$  chromanol 293B did not significantly increase APD. Our previ-

ous studies also demonstrated that the other  $I_{Ks}$  blocker, L-735,821 (100 nmol/L), did not significantly affect APD in the absence of a sympathetic agonist.

The explanation for these differences in results is unclear, although some investigators<sup>20</sup> have attributed them to differences between single-cell and multicellular preparations. Sun et al,<sup>21</sup> for instance, reported that higher chromanol concentrations (30 to 100  $\mu\text{mol/L}$ ) lengthened APD in perfused, multicellular dog ventricular muscle "wedge" preparations. From this finding, they speculated that chromanol is less able to diffuse into multicellular preparations than into single cells and that its effects are therefore less pronounced in multicellular preparations.<sup>22</sup> Thus, this group argues<sup>20,21</sup> that higher concentrations of chromanol are necessary in multicellular ventricular preparations to achieve  $I_{Ks}$  block and to increase APD than are necessary to completely block  $I_{Ks}$  in isolated myocytes. Other investigators also suggested that, because of its physical and/or chemical properties, L-735,821 poorly penetrates multicellular preparations but easily enters single myocytes (Dr J.J. Salata, unpublished personal communication, 1998).

These explanations appear unlikely. In the present study in human and in our previous studies in rabbit<sup>13</sup> and dog<sup>11</sup> ventricular muscle, a concentration of L-735,821 and HMR-1556 that fully blocked  $I_{Ks}$  in single myocytes also failed to increase APD in multicellular preparations, although L-735,821 and HMR-1556 are reportedly more specific and potent than chromanol 293B.<sup>23,24</sup> In addition, the L-735,821 and HMR-1556 concentrations used in our experiments, 100 nmol/L and 1  $\mu\text{mol/L}$ , respectively, are reported to be  $>10$  times their  $\text{EC}_{50}$  for  $I_{Ks}$  block.<sup>23,24</sup> Furthermore, concentrations of E-4031 and d-sotalol that block  $I_{Kr}$  in isolated myocytes markedly increased APD in multicellular preparations; it appears unlikely that either E-4031 or sotalol has distinctly different abilities to penetrate multicellular preparations and single myocytes than L-735,821.

On the other hand, Stengl et al<sup>14</sup> and Volders et al<sup>25</sup> recently obtained results similar to our finding that  $I_{Ks}$  block



does not affect normal ventricular muscle APD in species other than guinea pig in the absence of sympathetic stimulation. They reported that, in both dog ventricular myocytes and papillary muscle preparations, HMR-1556 (a highly selective  $I_{Ks}$  blocker) failed to lengthen APD without prior sympathetic stimulation,<sup>14,25</sup> even at high concentrations. These authors concluded that  $I_{Ks}$  block-induced repolarization lengthening requires an elevated degree of sympathetic tone as may occur in the setting of heart failure. Others<sup>26</sup> have suggested that the sensitivity of APD shortening by  $I_{Ks}$  block is enhanced when phosphorylation is increased as expected during increased sympathetic nerve activity. Clearly, cAMP increases  $I_{Ks}$  and may alter activation and deactivation kinetics for  $I_{Ks}$ . Thus,  $I_{Ks}$  is expected to have different effects on APD when sympathetic tone is increased. This relation between phosphorylation and the effects of  $I_{Ks}$  block on ventricular muscle APD needs further investigation to better elucidate the importance of sympathetic neural influences on electrogenesis in normal and diseased human myocardium.

Nonetheless, our present findings clearly indicate that neither chromanol 293B nor L-735,821 and HMR-1556 markedly affect normal human ventricular muscle APD over a normal range of heart rates in the absence of sympathetic stimulation. Thus,  $I_{Ks}$  in the absence of sympathetic neural agonists plays little role in the repolarization of normal ventricular muscle action potentials. Rather, in normal human ventricular myocardium,  $I_{Kr}$  is the outward current most responsible for termination of the action potential plateau and initiation of final action potential repolarization. However, as we have previously speculated,  $I_{Ks}$  may play a vital role in normal myocardium when APD is prolonged as after a pause in rhythm, decreased levels of thyroid hormone, or hypothermia. Some investigators suggest that  $I_{Ks}$  thus provides a "repolarization reserve" when other outward repolarizing currents are reduced, eg, by remodeling of ion currents during heart failure progression.<sup>27,28</sup> This role for  $I_{Ks}$  is supported by the finding that specific  $I_{Ks}$  block markedly lengthens APD after "repolarization reserve" is attenuated by  $I_{Kr}$  block with 50 nmol/L dofetilide. This observation is similar to that previously reported in dog ventricular muscle.<sup>11,29</sup>

The lack of effect of  $I_{Ks}$  on normal APD now found in human ventricular tissue in the absence of sympathetic stimulation and previously reported in dog<sup>11,29</sup> and rabbit<sup>13</sup> occurs because  $I_{Ks}$  is relatively small compared with  $I_{Kr}$  over the range of voltages encountered during the time course of normal ventricular action potentials, and its activation is slow compared with that of  $I_{Kr}$  in these species. Indeed we have shown that, in normal human ventricular myocytes,  $I_{Kr}$  in the absence of a sympathetic agonist activates rapidly ( $\tau=31.0\pm7.4$  ms;  $n=6$ ) during depolarizations to positive potentials (30 mV) but deactivates slowly at  $-40$  mV ( $\tau_1=599.9\pm53.9$  ms;  $\tau_2=6792.2\pm875.6$  ms;  $n=8$ ), whereas  $I_{Ks}$  activates slowly at positive potentials ( $\tau=1005\pm202.9$  ms at 30 mV;  $n=7$ ) and deactivates comparatively rapidly ( $\tau=132.4\pm29.8$  ms at  $-40$  mV;  $n=7$ ) with respect to diastolic intervals (300 to 700 ms) associated with physiological heart rates.<sup>7,12</sup>

These kinetics for  $I_{Ks}$  and  $I_{Kr}$  activation and deactivation indicate that, when APD is abnormally long or repolarization

reserves are attenuated and sympathetic activation is increased,  $I_{Ks}$  block should be expected to substantially lengthen APD while having little effect of normal APDs in the absence of sympathetic stimulation. Expectations were borne out when 10  $\mu$ mol/L chromanol 293B, 500 nmol/L HMR-1556, or 100 nmol/L L-735,821 was applied to normal dog ventricular muscle after APD was initially increased by exposure to E-4031 and veratrine<sup>11</sup> or by application of 100 nmol/L isoproterenol, which enhanced  $\beta$ -receptor activation and levels of intracellular cAMP.<sup>25</sup>

### Clinical Implications

Inherited mutations in  $I_{Ks}$  channel protein encoding are associated with the occurrence of LQT1,<sup>10,30</sup> yet our findings in normal human ventricular tissue in the absence of sympathetic stimulation indicate that pharmacological  $I_{Ks}$  block is not associated with an increase in APD, which is expected to correlate with an increase in long-QT duration. These 2 facts appear difficult to reconcile with one another, although it is known that penetrance (ie, the number of individuals having a particular genotype relative to those displaying the associated clinical phenotype) is rather low<sup>31,32</sup> in families with documented LQT1 mutations, histories of seizures, and sudden cardiac death. A far too simplistic explanation would be that individuals with mutated  $I_{Ks}$  channel proteins and elevated sympathetic tone lack the ability to compensate and limit excessive APD lengthening as a result of other causes such as extreme bradycardia, hypothyroidism, hypokalemia, changes in autonomic neural influences, or exposure to drugs affecting other repolarizing currents (eg,  $I_{Na,slow}$ ,  $I_{Cl}$ ,  $I_{to}$ ,  $I_{Kr}$ ,  $I_{K1}$ ). Although this question is not directly addressed by the study conducted, the results obtained suggest that if an increase in QT duration is the basis for an increased risk of arrhythmia and sudden cardiac death in people suffering from long-QT syndrome, then therapeutic interventions that increase "repolarization reserve" by any means should be equally effective in reducing QT duration in all forms of the long-QT syndrome.<sup>11,33,34</sup> An adequately powered, prospective clinical trial is required to address such speculation.

The findings reported in this study suggest that antiarrhythmic drugs that selectively block  $I_{Ks}$  are unlikely to affect ventricular arrhythmias in the absence of sympathetic neural stimulation. However, it must be recognized that sympathetic tone is forever fluctuating in the in situ human heart, and many believe that the selective  $I_{Ks}$  block in combination with  $\beta$ -adrenoceptor blockade should have antiarrhythmic benefit, citing the clinical antiarrhythmic effectiveness of amiodarone, which has both  $I_{Ks}$  and  $\beta$ -adrenoceptor blocking properties during chronic administration.<sup>35</sup> Nonetheless, another little explored antiarrhythmic strategy might be to increase, rather than to block,  $I_{Ks}$ . If  $I_{Ks}$  were increased (either pharmacologically or genetically), arrhythmia risk might be expected to be lowered; certainly, such an antiarrhythmic intervention would benefit patients with inherited or acquired long-QT syndrome. Therapeutic increases in  $I_{Ks}$  would increase repolarization reserve and possibly reduce the risk of sudden cardiac death during progression of heart failure when  $I_{Kr}$  and  $I_{to}$  expression are downregulated.<sup>27,28,33,34</sup>

## Conclusions

We earlier reported that in dog and rabbit ventricular myocytes,  $I_{Ks}$  plays no obvious role in altering action potential repolarization and QT duration at normal heart rates. In the present study, in the absence of sympathetic stimulation, this finding is confirmed in isolated human ventricular preparations obtained from the hearts of individuals without heart disease. These findings should not be misconstrued as meaning that  $I_{Ks}$  does not play an important role in the normal heart where sympathetic stimulation is ever present and fluctuating continuously. We also believe that  $I_{Ks}$  is vitally important in the normal heart where it prevents excessive action potential prolongation in the setting of an elevated sympathetic tone after a single long diastolic interval after the compensatory pause that follows a premature ventricular depolarization or during bradycardia or when APD is prolonged by other means (eg, by unintentional  $I_{Kr}$  block, hypothyroidism, or serum hypokalemia).

## Acknowledgments

This work was supported by grants from the Hungarian National Research Foundation (OTKA T-035018, T-037520, and T-048698), Hungarian Ministry of Health (T-188/2003), and Hungarian Ministry of Education (Bio-37 KPI); National Research and Development Programmes (NKFP 1A/0011/2002) from the Hungarian Academy of Sciences; and the János Bolyai Research Scholarship (Drs Jost and Virág). Dr Lathrop participated in an uncompensated capacity on his own time, and although he is employed by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health within the US Department of Health and Human Services, no support was received, and no endorsement should be inferred.

## References

- Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier  $K^+$  current: differential sensitivity to block by class III antiarrhythmic agents. *J Gen Physiol*. 1990;96:195–215.
- Gintant GA. Two components of delayed rectifier current in canine atrium and ventricle: does  $I_{Ks}$  play a role in the reverse rate dependence of class III agents? *Circ Res*. 1996;78:26–37.
- Salata JJ, Jurkiewicz NK, Jow B, Folander K, Guinasso PJ, Raynor B, Swanson R, Fermini B.  $I_K$  of rabbit ventricle is composed of two currents: evidence for  $I_{Ks}$ . *Am J Physiol*. 1996;271:H2477–H2489.
- Beuckelmann DJ, Näbauer M, Erdmann E. Alteration of  $K^+$  currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res*. 1993;73:379–385.
- Veldkamp MW, Van Ginneken ACG, Ophof T, Bouman LN. Delayed rectifier channels in human ventricular myocytes. *Circulation*. 1995;92:3497–3504.
- Li GR, Feng J, Yue L, Carrier M, Nattel S. Evidence for two components of delayed rectifier  $K^+$  current in human ventricular myocytes. *Circ Res*. 1996;78:689–669.
- Iost N, Virág L, Opincariu M, Szécsi J, Varró A, Papp JG. Delayed rectifier potassium current in undiseased human ventricular myocytes. *Cardiovasc Res*. 1998;40:508–515.
- Hondeghem LM, Snyders DJ. Class III antiarrhythmic agents have a lot of potential but a long way to go: reduced effectiveness and dangers of reverse use dependence. *Circulation*. 1990;81:686–690.
- Hohnloser SH, Woosley RL, Sotalol. *N Engl J Med*. 1994;331:31–38.
- Roden DM, Lazzara R, Rosen M, Schwartz PJ, Towbin J, Vincent GM, for the SADS Foundation Task Force on LQTS. Multiple mechanisms in the long QT syndrome: current knowledge, gaps, and future directions. *Circulation*. 1996;94:1996–2012.
- Varró A, Baláti B, Iost N, Takács J, Virág L, Lathrop DA, Lengyel C, Tólosi L, Papp JG. The role of the delayed rectifier component  $I_{Ks}$  in dog ventricular muscle and Purkinje fibre repolarization. *J Physiol*. 2000;523:1:67–81.
- Virág L, Iost N, Opincariu M, Szolnoky J, Szécsi J, Bogáts G, Szenohradszky P, Varró A, Papp JG. The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes. *Cardiovasc Res*. 2001;49:790–797.
- Lengyel Cs, Iost N, Virág L, Varró A, Lathrop AD, Papp JG. Pharmacological block of the slow component of the outward delayed rectifier current ( $I_{Ks}$ ) fails to lengthen rabbit ventricular muscle  $QT_c$  and action potential duration. *Br J Pharmacol*. 2001;132:101–110.
- Stengl M, Volders PG, Thomsen MB, Spatjens RL, Sipido KR, Vos MA. Accumulation of slowly activating delayed rectifier potassium current ( $I_{Ks}$ ) in canine ventricular myocytes. *J Physiol*. 2003;551:777–786.
- Hund TJ, Rudy Y. Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model. *Circulation*. 2004;110:3168–3174.
- Iost N, Virág L, Opincariu M, Szécsi J, Varró A, Papp JG. Does  $I_{Ks}$  play an important role in the repolarization in normal human ventricular muscle? *Circulation*. 1999;100(suppl 1):I-495. Abstract.
- Isenberg G, Klockner U. Calcium tolerant ventricular myocytes prepared by preincubation in a “KB medium.” *Pflugers Arch*. 1982;395:6–18.
- Bosch RF, Gaspo R, Busch AE, Lang HJ, Li GR, Nattel S. Effects of the chromanol 293B, a selective blocker of the slow component of the delayed rectifier  $K^+$  current, on repolarisation in human and guinea pig ventricular myocytes. *Cardiovasc Res*. 1998;38:441–450.
- Schrieck J, Wang Y, Gjini V, Korth M, Zrenner B, Schomig A, Schmitt C. Differential effect of  $\beta$ -adrenergic stimulation on the frequency-dependent electrophysiologic actions of the new class III antiarrhythmics dofetilide, ambasilide, and chromanol 293B. *J Cardiovasc Electrophysiol*. 1997;8:1420–1430.
- Sun ZQ, Thomas G, Antzelevitch C. Role of the delayed rectifier component  $I_{Ks}$  in cardiac repolarization. *J Cardiovasc Electrophysiol*. 2001;10:1205–1206. Reply.
- Sun ZQ, Thomas GP, Antzelevitch C. Chromanol 293B inhibits slowly activating delayed rectifier and transient outward currents in canine left ventricular myocytes. *J Cardiovasc Electrophysiol*. 2001;12:472–478.
- Shimizu W, Antzelevitch C. Cellular basis for the ECG features of the LQT1 form of the long-QT syndrome: effects of  $\beta$ -adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarisation and torsade de pointes. *Circulation*. 1998;98:2314–2322.
- Salata JJ, Jurkiewicz NK, Sanguinetti MC, Siegl PK, Claremon DA, Remy DC, Elliot JM, Libby BE. The novel class III antiarrhythmic agent, L-735821, is a potent and selective blocker of  $I_{Ks}$  in guinea pig ventricular myocytes. *Circulation*. 1996;94:3095. Abstract.
- Thomas GP, Gerlach U, Antzelevitch C. HMR 1556, a potent and selective blocker of slowly activating delayed rectifier potassium current. *J Cardiovasc Pharmacol*. 2003;41:140–147.
- Volders PG, Stengl M, van Opstal JM, Gerlach U, Spatjens RL, Beekman JD, Sipido KR, Vos MA. Probing the contribution of  $I_{Ks}$  to canine ventricular repolarization: key role for  $\beta$ -adrenergic receptor stimulation. *Circulation*. 2003;107:2753–2760.
- Han W, Wang Z, Nattel S. Slow delayed rectifier current and repolarization in canine cardiac Purkinje cells. *Am J Physiol*. 2001;280:H1075–H1080.
- Kaab S, Nabauer M. Diversity of ion channel expression in health and disease. *Eur Heart J Suppl* 3. 2001;K:K31–K40.
- Volders PG, Sipido KR, Vos MA, Spatjens RL, Leunissen JD, Carmeliet E, Wellens HJ. Downregulation of delayed rectifier  $K^+$  currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes. *Circulation*. 1999;100:2455–2461.
- Biliczki P, Virág L, Iost N, Papp JG, Varró A. Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of repolarization reserve. *Br J Pharmacol*. 2002;137:361–368.
- Wang Z, Tristani-Firouzi M, Xu Q, Lin M, Keating MT, Sanguinetti MC. Functional effects of mutations in KvLQT1 that cause long QT syndrome. *J Cardiovasc Electrophysiol*. 1999;10:817–826.
- Priori SG, Napolitano C, Bloise R, Schwartz P. Low penetrance in the long QT syndrome: the importance of molecular diagnosis. *Eur Heart J*. 1998;19(suppl):424. Abstract.
- Swan H, Saarinen K, Kontula K, Toivonen L, Viitasalo M. Evaluation of QT interval duration and dispersion and proposed clinical criteria in diagnosis of long QT syndrome in patients with a genetically uniform type of LQT1. *J Am Coll Cardiol*. 1998;32:486–491.
- Xu XP, Salata JJ, Wang JX, Wu Y, Yan GX, Liu TX, Marinchak RA, Kowey PR. Increasing  $I(Ks)$  corrects abnormal repolarization in rabbit models of acquired LQT2 and ventricular hypertrophy. *Am J Physiol*. 2002;283:H664–H670.
- Salata JJ, Jurkiewicz NK, Wang JX, Evans BE, Orme HT, Sanguinetti MC. A novel benzodiazepine that activates cardiac slow delayed rectifier  $K^+$  currents. *Mol Pharmacol*. 1998;54:220–230.
- Kamiya K, Nishiyama A, Yasui K, Hojo M, Sanguinetti MC, Kodama I. Short- and long-term effects of amiodarone on the two components of cardiac delayed rectifier  $K^+$  current. *Circulation*. 2001;103:1317–1324.